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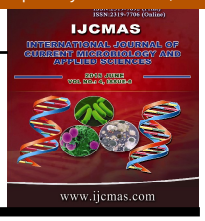

Original Research Article

Efficacy of Plant Extracts against the Larvae of Filariasis Vector, *Culex quinquefasciatus* Say and the Dengue Vector *Aedes aegypti* Linn at Mysore

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K e y w o r d s

Aedes aegypti, *Culex quinquefasciatus*, Efficacy, *Heracleum rigens*, Larvicide and susceptible

A B S T R A C T

Mosquito control has been facing backlashes because of the emergence of resistant varieties against synthetic insecticides. Hence biologically active environment friendly insecticides of plant origin have received renewed attention in recent years as agents for vector control**.** The present investigation highlights the larvicidal efficacy of petroleum ether, chloroform, ethyl acetate, methanol and acetone extracts of *Heracleum rigens* seeds against 2nd, 3rd and 4th instar larvae of the laboratory reared *Culex quinquefasciatus* and *Aedes aegypti*. The larval susceptibility tests were conducted following the WHO standard procedure (WHO, 2005). The LC_{50} values of *Heracleum rigens* for *Culex quinquefasciatus* and *Aedes aegypti* respectively are 7.19 and 40.64 ppm against 2nd instar; 24.80 and 91.55 ppm against 3rd instar and 69.25 and 113.69 ppm against 4th instar larvae. The results suggest that 2nd instar larvae were significantly susceptible compared to 3rd and 4th instar larvae and that *Heracleum rigens* may contain promising larvicides against different mosquito species.

Introduction

Mosquito species are well known as vectors of human diseases particularly malaria, lymphatic filariasis, dengue, chikungunya, Japanese encephalitis and yellow fever. Malaria and lymphatic filariasis rank amongst the world's most prevalent tropical vector borne communicable diseases (Abdalla *et al.*, 2009). Lymphatic filariasis caused by *Wuchereria bancrofti* and transmitted by *Culex quinquefasciatus* is found to be more endemic in the Indian subcontinent (Rajasekaraiah *et al.*, 1991). *Aedes aegypti* is the principal vector of dengue fever and dengue hemorrhagic fever in urban and semi urban areas of the tropics worldwide. Chikungunya and yellow fever are other diseases transmitted by this mosquito (Soboka and Styczynska, 1991). The systematic application of insecticide is a common and widely accepted approach to control mosquito population, as it w provides rapid solution. Such chemical control measures although highly effective, is still facing a threat, as selective imposed by conventional insecticides is enhancing resistance in various mosquito species

resulting in disease outbreaks (Liu *et al.*, 2007). It has also resulted in undesirable effects on non-target organisms and fostered environmental and human health (Brown, 1986).

In this regard, natural products of plant origin with the insecticidal property have been tried in the recent past for the control of various insect pests and vectors. The botanical pesticides are generally target-specific, readily biodegradable and usually lack toxicity to higher animals (Bowers, 1992). Such plant derivatives may act as larvicides, insect growth regulators, repellents and oviposition attractants and thereby play an important role in the interruption of the transmission of mosquito-borne diseases (Babu and Murugan, 1998; Bagavan *et al.*, 2008; Ghosh *et al.*, 2008; Kannathasan *et al.*, 2008; Prathibha *et al.*, 2010). The assessment of the efficacy of different phytochemicals obtained from various local plants is the best way to develop novel synthetic insecticides (Sukumar *et al.*, 1991). In the light of the above knowledge, a few local plants were screened at Mysore and extracts of the species *Heracleum rigens* was selected for bioassay in the present investigation. *Heracleum rigens* is a medicinal plant, found widespread in southern peninsular India, Western Ghats, interior districts of Karnataka and Tamil Nadu. The seed oil of this is used to treat urinary disorders, hyperacidity, cardiac diseases, wounds, abdominal disorders and indigestion, diarrhea and headache (Yoganarasimhan, 1996).

Materials and Methods

The seeds of *H. rigens* were purchased from shops at Hassan town, Karnataka, India. After proper identification, seeds were dried under shade for 8–10 days at room temperature of 25±2ºC, powdered mechanically with the help of a laboratory hand blender. This product was then subjected to extraction with petroleum ether, chloroform, ethyl acetate, methanol and acetone using a Soxhlet extractor to obtain crude extracts. The extracts so obtained were allowed to dry and transferred to air tight bottles to be stored in refrigerator until required for conducting larval bioassay. Larvae of *Cx. quinquefasciatus* and *Ae. Aegypti* were procured from the colony kept at Vector Biology Research Lab, Department of Zoology, where the experiments have been carried out.

Larval Bioassay

The larval susceptibility tests were conducted following the WHO standard procedure (WHO, 2005). Different concentrations of the extracts were prepared by serial dilutions of stock solution using acetone as solvent. Group of 25 late 3rd or early 4th instar larvae were released into the glass beakers of 500ml capacity containing 249ml dechlorinated tap water to which 1ml of extract was added. The toxicity of each extract was determined with five different concentrations. Control beakers contained 249ml dechlorinated tap water with 25 larvae and 1ml of acetone. Control and test beakers were maintained at same conditions at 25±2ºC, 12h light/dark regime. All the treatments were repeated four times. The larvae were considered as dead or moribund, if not responsive to gentle prodding with a fine needle.

Statistical analysis

Mortality counts were taken after 24h exposure. The larvae that have pupated during the test were discarded. The test with control mortality of over 20% was considered unsatisfactory and in such cases,

the experiment was repeated. The average larval mortality data were subjected to probit analysis. LC_{50} values were considered to be significantly different if the 95% Fiducial limits of two LC_{50} values did not overlap each other (Yang *et al.,* 2002). For calculating $LC_{50,}$ LC_{90} and 95% Fiducial limits of upper confidence limit, lower confidence limit and the statistical package for the social sciences (SPSS) software was employed.

Results and Discussion

The data on the larvicidal efficacy of crude extracts of *Heracleum rigens* seeds tested against the 2nd, 3rd and 4th instar larvae of *Culex quinquefasciatus* and *Aedes aegypti* are provided in table 1 and 2 and also depicted in figure 1 and 2.

Table-1 provides the data on the efficacy of petroleum ether, chloroform, ethyl acetate, methanol and acetone extracts of *H. rigens* seeds against *Cx. quinquefasciatus* larvae with LC_{50} values being 7.19, 11.98, 14.50, 22.11 and 28.40ppm respectively against second instar as against 24.80, 46.93, 57.84, 70.09 and 77.65 ppm respectively against third instar larvae. Likewise 69.25, 92.61, 115.08, 165.94 and 215.10 ppm are the LC_{50} values for the said extracts in sequence against fourth instar larvae.

Similarly Table-2 gives the data on the efficacy of petroleum ether, chloroform, ethyl acetate, methanol and acetone extracts of *H. rigens* seeds against *Ae. aegypti* larvae with LC_{50} values being 40.64, 69.22, 70.65, 74.70 and 97.07 ppm respectively against second instar and 91.55, 114.25, 143.48, 195.57 and 234.77 ppm against third instar larvae. The LC_{50} values against fourth instar larvae were found to be 113.69, 144.64, 165.43, 231.26 and 308.65 ppm respectively.

Second instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* were significantly susceptible with LC_{50} value 7.19 ppm and 40.64 ppm respectively ($p<0.05$). Among the five solvents used petroleum ether was found to be more effective in extracting larvicidal compounds from the plant. The log dose probit mortality response of two species is provided in Figure 1 and 2.

Mosquito borne diseases are one of the most serious public health problems in the developing countries. It can be controlled to a large extent by preventing mosquito bites using repellents, larvicides and adulticides. Environmental safety of insecticide is considered to be of paramount importance while employing against pests and vectors. In this regard, screening of locally available plant species for mosquito control will reduce dependence on expensive imported products and stimulate local efforts to enhance public health (Bowers *et al.,* 1992). Thus the present study showed that all the five organic solvent extracts obtained from *H. rigens* seeds have shown larvicidal activity against *Culex* and *Aedes* species at Mysore. However the petroleum ether extracts was found to be more effective followed by chloroform, ethyl acetate, methanol and acetone. The purpose of a general screening for bioactivity is to extract as many potentially active constituents as possible. This is achieved by using solvents ranging from water with a polarity index (P) of 10.2 to hexane is 0.1 including number of intermediary solvents such as methanol (6.1), acetone (5.1), ethyl acetate (4.4) chloroform (4.1) and petroleum ether. The data on *H. rigens* seeds extracts indicates that converse relationship between extract efficacy and solvent polarity where efficacy increase with decreasing polarity. This is in line with the observation made by Aivazi and Vijayan (2009), in oak gall extract at Mysore.

Table.1 Larvicidal activity of different solvent extracts of *Heracleum rigens* against larvae of *Culex quinquefasciatus*

Species	Instars	Extraction solvents	LC_{50} ±SE ppm (LCL-UCL)	LC_{90}± SE ppm (LCL-UCL)	Regression equation	X^2 (df)	significance
Culex quinquefasciatus	*2nd instar	Petroleum ether	**7.19**±0.183 (3.64-10.82)	15.62 ±0.183 (10.49 - 97.81)	Y = 3.8075 X ±1.7365	23.74(3)	*0.025
		Chloroform	**11.98** ±0.063 (11.34-12.66)	20.83 ± 0.063 (18.98- 23.59)	Y = 5.3369 X±0.7571	4.99(3)	*0.049
		Ethyl acetate	**14.50** ±0.129 (11.96-17.36)	23.34 ±0.129 (18.92- 44.79)	Y = 6.1989 X ±2.2002	12.84(3)	*0.044
		Methanol	**22.11** ±0.064 (21.11- 23.08)	34.14±0.064 (31.99- 37.13)	Y = 6.7929 X±4.1339	0.39(3)	*0.038
		Acetone	**28.40** ± 0.066 (27.50- 29.30)	38.46 ± 0.066 (36.73- 40.77)	Y = 9.7343 X ±9.1481	4.37(3)	*0.129
	3rd instar	Petroleum ether	**24.80** ±0.147 (15.95- 34.20)	55.14 ± 0.147 (38.57- 175.24)	Y = 3.6931 X ±0.1499	15.85 (3)	*0.041
		Chloroform	**46.93** ± 0.065 (45.04 -48.82)	69.56 ± 0.065 (65.48- 75.18)	Y = 7.4970 X ±7.5309	2.21(3)	0.754
		Ethyl acetate	**57.84** ± 0.062 (55.66-60.05)	85.43 ± 0.062 (80.14- 93.06)	Y = 7.5679 X ±8.3369	6.42(3)	0.270
		Methanol	**70.09** ± 0.063 (67.97- 72.29)	95.93 ± 0.063 (90.99-102.93)	Y = 9.4029 X ±12.3548	2.17(3)	*0.037
		Acetone	**77.65** ±0.106 (71.09-84.17)	103.01 ±0.106 (92.72- 131.65)	Y = 10.4450 X±14.7431	8.63(3)	*0.017
	4th instar	Petroleum ether	**69.25** ± 0.161 (51.04-86.34)	114.51 ± 0.161 (90.64- 235.53)	Y = 5.8675 X ±5.7990	17.91(3)	**0.001
		Chloroform	**92.61**±0.103 (77.52-108.69)	166.69 ±0.103 (134.87- 267.98)	Y = 5.0207 X ±4.8740	8.02(3)	**0.001
		Ethyl acetate	**115.08** ±0.062 (109.39-120.75)	191.07 ±0.062 (76.00- 213.56)	Y = 5.8200 X ±6.9951	2.53 (3)	**0.001
		Methanol	**165.94** ± 0.064 (160.90-170.90)	224.02 ± 0.064 (213.86-237.98)	Y = 9.8331 X ±16.8291	2.80(3)	**0.001
		Acetone	**215.10** ± 0.065 (205.64-224.70)	331.70±0.065 (309.98- 361.95)	Y = 6.8134 X ±10.8933	3.22 (3)	**0.001

LC_{50}=Median lethal concentration, LC_{90}= 90% lethal concentration, LCL=Lower confidence limit, UCL=Upper confidence limit df= degree of freedom
*The difference in LC_{50} is significant based on the non overlapping of 95% Fiducial limit ($P<0.05$)
**The difference in LC_{50} is significant based on the non overlapping of 95% Fiducial limit ($P<0.01$)

Table.2 Larvicidal activity of different solvent extracts of *Heracleum rigens* against larvae of *Aedes aegypti*

Species	Instars	Extraction solvents	LC_{50} ±SE (ppm LCL-UCL)	LC_{90}± SE (ppm LCL-UCL	Regression equation	X^2 (df)	significance
Aedes aegypti	2nd instar	Petroleum ether	**40.64**± 0.11 (34.52- 46.63)	65.49 ± 0.11 (55.09- 95.74)	Y = 6.1839 X ±4.9497	8.98 (3)	0.288
		Chloroform	**69.22**± 0.0638 (64.88- 73.83)	132.95± 0.063 (119.05- 154.13)	Y = 4.5213 X ±3.3204	6.66(3)	**0.001
		Ethyl acetate	**70.65**± 0.0645 (68.65 - 72.61)	93.11 ±0.064 (89.27- 98.33)	Y = 10.6931 X ±14.7734	0.13(3)	**0.001
		Methanol	**74.70±** 0.1750 (48.40 - 106.23	135.07 ± 0.175 (98.47-784.27)	Y = 4.9825 X ±4.3340	23.12(3)	0.066
		Acetone	**97.07**± 0.21	198.99 ±0.217	Y = 4.1113 X ±3.1697	38.20(3)	0.066
	3rd instar	Petroleum ether	**91.55**± 0.102 (77.00- 106.71)	162.09±0.102 (132.51- 250.73)	Y = 5.1662 X ±5.1344	7.85 (3)	**0.001
		Chloroform	**114.25**± 0.063 (109.04 -119.40)	179.99 ±0.063 (167.73-197.49)	Y = 6.4927 X ±8.3611	6.34(3)	**0.001
		Ethyl acetate	**143.48**± 0.109 (127.39 - 159.76)	207.45 ±0.109 (180.71-285.42)	Y = 8.0042 X ±12.2636	8.94(3)	**0.001
		Methanol	**195.57**± 0.063 (184.74 - 206.95)	348.56± 0.063 (316.37-396.79)	Y = 5.1065 X ±6.7005	2.02(3)	*0.002
		Acetone	**234.77** ± 0.125 (194.08-276.80)	-	Y = 6.2271 X ±9.7623	12.01(3)	**0.001
	4th instar	Petroleum ether	**113.69**± 0.065 (108.89-118.43)	171.12±0.065 (160.89-185.23)	Y = 7.2174 X ±9.8371	7.67(3)	**0.001
		Chloroform	**144.64**± 0.063 (139.42-149.92)	209.16 ±0.063 (197.09-226.24)	Y = 8.0008 X ±12.2843	5.11(3)	**0.001
		Ethyl acetate	**165.43**± 0.062 (159.99- 170.76)	230.21±0.062 (218.49-246.77)	Y = 8.9302 X ±14.8128	3.57(3)	**0.001
		Methanol	**231.26**± 0.063 (220.94- 241.54)	361.68 ±0.063 (337.28-396.43)	Y = 6.5983 X ±10.5991	3.74(3)	**0.001
		Acetone	**308.65**± 0.140 (229.00- 391.32)	577.14 ±0.140 (440.53-1242.31)	Y = 4.7149 X ±6.7375	14.05(3)	**0.001

LC_{50}=Median lethal concentration, LC_{90}= 90% lethal concentration, LCL=Lower confidence limit, UCL=Upper confidence limit df= degree of freedom
*The difference in LC_{50} is significant based on the non overlapping of 95% Fiducial limit (P<0.05)
**The difference in LC_{50} is significant based on the non overlapping of 95% Fiducial limit (P<0.01)

Fig.1 Larvicidal activity of different solvent extracts of *Heracleum rigens* against 2nd instar larvae of *Culex quinquefasciatus*

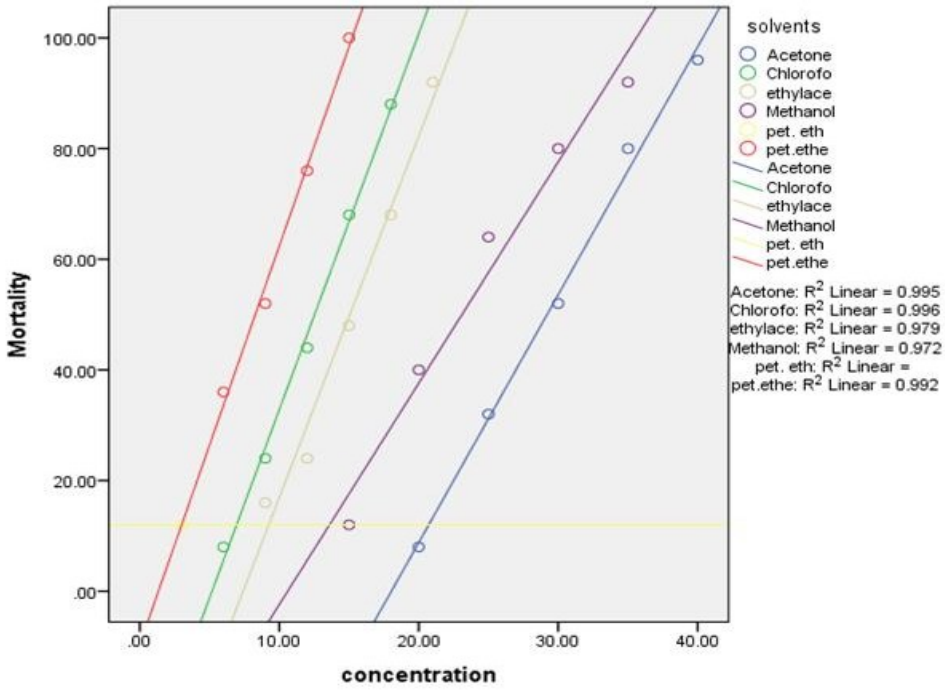
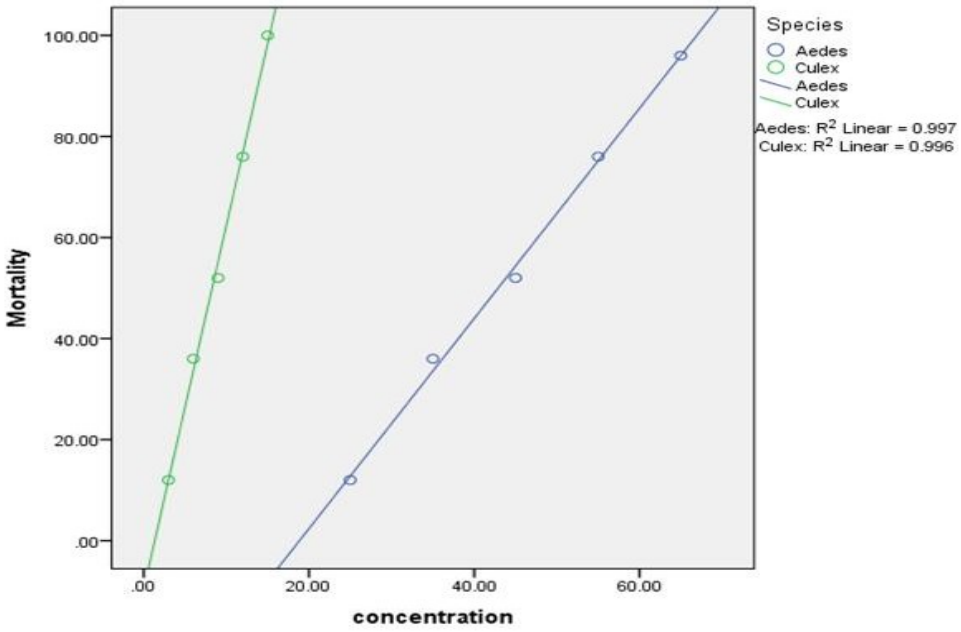


Fig.2 Effect of *Heracleum rigens* petroleum ether extract on 2nd instar larvae of *Culex quinquefasciatus* and *Aedes aegypti*



Apiaceae family taxa with species such as *Heracleum sphondylium, Seseli montanum, Conopodium capillifolium, Bupleurum fruticosum, Oenanthe pimpinelloides* and *Eleoselinum aselepium* have also been found to display good larvicidal activity (Evergetis *et al.*, 2009). The seed extracts of *Apium graveolens*, *Pimpinella anisum and Ammi visnaga* exhibited larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* (Momin and Nair, 2001; Prajapati *et al.*, 2005; Pavela, 2008). The fruit extracts of *Carum carvi*, *Daucus carota*, *Ferula galbaniflua* and *Angelica archangelica* too showed larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* (Lee, 2006; Amer and Mehlhorn, 2006; Pavela, 2009). The stem extacts of *Ferula lancerottensis, Ferula assa-foetida, Seseli tortuosum and Seseli pallasii* have shown larvicidal activity against *Culex quinquefasciatus* (Pavela, 2008; Pavela, 2009). In line with the above results, the present study with *H. rigens* extracts exhibited good larvicidal activity against two mosquito species. However second instar larvae were significantly sensitive than third and fourth instars. *Culex quinquefasciatus* larvae showed more susceptibility than *Aedes aegypti*. Thus, extracts of *Heracleum rigens* seeds may be a promising source for characterizing bioactive compounds for conducting further tests on mosquito control. As it is an indigenous and locally available plant, continuation of the investigations is underway.

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